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MECHANOTRANSDUCTION

The Mechanotransduction Machinery of Hair Cells

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Mechanotransduction, the conversion of mechanical force into an electrochemical signal, allows living organisms to detect touch, hear, register movement and gravity, and sense changes in cell volume and shape. Hair cells in the vertebrate inner ear are mechanoreceptor cells specialized for the detection of sound and head movement. Each hair cell contains, at the apical surface, rows of stereocilia that are connected by extracellular filaments to form an exquisitely organized bundle. Mechanotransduction channels, localized near the tips of the stereocilia, are gated by the gating spring, an elastic element that is stretched upon stereocilia deflection and mediates rapid channel opening. Components of the mechanotransduction machinery in hair cells have been identified and several are encoded by genes linked to deafness in humans, which indicates that defects in the mechanotransduction machinery are the underlying cause of some forms of hearing impairment.

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Presentation Notes

Slide 1: Science Signaling *logo*

The slideshow and notes for this presentation are provided by *Science Signaling* (www.sciencesignaling.org).

Slide 2: The mechanotransduction machinery of hair cells

This presentation focuses on recent discoveries that have shed light on the composition of the mechanotransduction machinery of hair cells in the mammalian inner ear.

Slide 3: Mechanotransduction and perception

Mammals contain various sensory cells that are activated by mechanical signals. These include muscle spindles and Golgi tendon organs for sensing skeletal muscle stretch; skin sensory neurons for perceiving texture, vibration, and pressure; and hair cells in the inner ear for perceiving sound and head movements. Each of these cells is specialized for the conversion of mechanical stimuli into electrical signals, a process that is known as mechanotransduction.

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Slide 4: Modeling mechanotransduction

A simple blueprint has been proposed that might explain how mechanosensory cells convert mechanical stimuli into electrical signals. In this model, an ion channel is directly gated by mechanical force, leading to depolarization of the receptor cell and the subsequent propagation of the electrical signals throughout the nervous system. Transduction channels have been proposed to be tethered intracellularly and extracellularly to achieve sensitivity to mechanical force. Deflection relative to the anchor points changes the conformation of the transducer channel, thus leading to changes in ion influx.

Slide 5: Mechanotransduction in C. elegans

Support for this simple blueprint has been obtained from studies of mechanotransduction in the nematode *Caenorhabditis elegans*, in which touch is communicated by mechanosensitive neurons that lie below the proteinaceous cuticle secreted by the epidermis. Several components of the mechanotransduction machinery have been identified in screens for mutations that suppress response to light touch. These findings provide evidence that the transduction channel in the sensory neurons is tethered intracellularly to the microtubular cytoskeleton and extracellularly to components of the extracellular matrix (*1*).

Slide 6: The mammalian inner ear: The cochlea

The sensory epithelium for the perception of sound in mammals is situated within the snail-shaped cochlea of the inner ear, as shown in this illustration adapted from Holley (*2*). Auditory mechanoreceptor cells along the length of the cochlea vary in their frequency sensitivity: Cells that respond to the highest frequencies are located at the base of the cochlea, and those that respond to the lowest frequency are at the apex. This tonotopic organization very much resembles the organization of the keys in a piano.

Slide 7: The mammalian inner ear: The hair cells

A section through a cochlea reveals the hair cells (represented in blue), which are the mechanoreceptors for the perception of sound. These cells were named for the bundles of stereocilia that project from the apical cell surfaces to form the mechanoreceptive organelle. The cochlear sensory epithelium contains one row of inner hair cells (IHCs) and three rows of outer hair cells (OHCs). The principal function of OHCs is to amplify mechanical inputs, whereas IHCs receive most of the afferent innervation and transmit sound information to the central nervous system.

Slide 8: Hair bundles and mechanotransduction

The diagram illustrates a section through the hair bundle of a hair cell, in which stereocilia are organized in rows of increasing height and are interconnected by fibrous linkages, such as the top connectors and tip links. Tip links connect the tips of stereocilia to their next taller neighbors and project in an oblique angle. Top connectors sit below the tips of stereocilia and connect adjacent stereocilia but do not project in an oblique angle. The longest stereocilia of OHCs are in direct contact with an extracellular matrix assembly known as the tectorial membrane. Sound vibrations that deflect the hair bundle toward the longest stereocilia lead to an influx of cations into the stereocilia and subsequent depolarization of the hair cell.

Several lines of evidence support the model that tip links are required for channel gating. First, tip links are aligned parallel to the direction of the mechanical sensitivity of the hair bundle. Second, when tip links are dis-

rupted by treatment with calcium chelators or proteases, mechanotransduction ceases. Third, upon mechanical stimulation, cations enter the stereocilia near their tips. The graph on the right shows a typical current trace recorded from a hair cell. Mechanical deflection induces a rapid inward current, which subsequently declines. The decline, referred to as adaptation, occurs on both a fast and slow time constant. Adaptation ensures that the sensitivity of the hair bundle to mechanical stimulation is maintained.

Slide 9: The tip-link model

These diagrams illustrate some general features of the prevailing tip-link model of channel gating, incorporating recent findings by the Ricci laboratory, which have provided compelling evidence that the mechanotransduction channels are localized in proximity to the lower tip-link insertion site (*3*). According to the current model, the lower end of the tip link is connected directly or indirectly to the transduction channel, and the upper end is connected to an "adaptor" consisting of a cluster of myosin 1c (MYO1C) molecules. Deflection of the hair bundle increases tension in the tip link, which has been proposed to act as the transduction channel gating spring itself or to be in line with it, leading to rapid channel opening. Increased tension leads to slippage of the MYO1C motor complex down the cytoskeleton, relaxing the tension and leading to channel closure. Subsequently, the MYO1C motor complex climbs up again, reestablishing tension.

Slide 10: Hearing impairment and deafness

The study of the inner ear is important not only for elucidating the mechanisms that control hair cell function, but also for identifying defects in hair cells that cause hearing loss. Hearing loss is the most common form of sensory impairment in humans: 1 in 1000 children is born with hearing impairment, and 16% of adults suffer from some degree of hearing loss. The likelihood that individuals will suffer from hearing loss increases with age, with 25% of the population above age 65, and 80% of that above age 80, being affected.

In addition to environmental risk factors, such as exposure to noise and chemicals, hearing loss is frequently of genetic origin (*4*). It is estimated that mutations in any one of more than 400 genes can lead to deafness, but only a fraction of the affected genes have been identified. These encode proteins with various functions and include cell adhesion molecules, cytoskeletal components, and transcription factors. These molecules are critical not only for understanding the etiology of the disease, but also for defining the mechanisms that control hair cell development and function.

Slide 11: Genetic screen for recessive deafness traits

Although the tip-link model of transducer channel gating was proposed more than 25 years ago, it has been very difficult to identify components of the auditory mechanotransduction machinery because hair cells are relatively inaccessible and few in number. The observation that gene mutations are a frequent cause of hearing loss in humans prompted us to take a genetic approach to identify genes that might encode components of the mechanotransduction machinery of hair cells. We carried out an *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screen in mice to identify recessive mutations that affect hair cell function and cause deafness (*5*). To identify mice with auditory defects, we first inspected them visually and performed behavioral tests to exclude lines with obvious developmental defects or general nervous system dysfunction. Mice that did not show obvious defects were subsequently analyzed for the auditory startle response, which analyzes locomotor activity in response to an auditory stimulus. Mice that had a defect in the auditory startle response were then analyzed by measuring the auditory brainstem response (ABR), which is a readout for the transmission of electrical currents along the auditory pathway from the activation of the auditory afferent neurons that form synapses with hair cells through relays within the central nervous system and to the auditory processing center of the brain.

Stimulation with decreasing sound intensities from 90 dB to 35 dB can be used to determine the auditory threshold, the lowest sound level an animal can detect. Representative ABR traces from wild-type (top) and deaf mutant (bottom) mice are shown. The auditory threshold in the control C57Bl/6 mouse was at or below 45 dB; the mutant mouse did not respond to any sound.

Slide 12: New deaf mouse lines

This table summarizes the current status of our ongoing screen in which mice were screened for both hearing and balance defects. Defects in hair cells frequently lead to balance defects in addition to hearing impairment because hair cells are also found in the vestibule, an inner ear structure that detects head movement. Thus far we have identified 20 lines with recessive deafness traits. These lines were named for Latin dances when the animals were deaf and for folk dances when deafness was associated with balance defects (as manifested by circling behavior and inability to swim).

We mapped and positionally cloned several of the affected genes. Seven of these had been previously associated with hearing loss (*VLGR1*, *otoferlin*, *Myo6*, *Myo7a*, *pejvakin*, *CDH23*), but three had not been linked to deafness, including the *COMT2* gene, which had not previously been identified by sequence analysis of the mouse or human genome (*6*). Subsequent studies showed that mutations in the human orthologs of the three mouse genes also cause deafness, reinforcing the assumption that the mouse is a good model for studying human deafness. Furthermore, all the identified genes are expressed in hair cells, providing evidence that our screen is useful for identifying genes that regulate hair cell function and possibly mechanotransduction. Notably, the three genes affected in four of the mutant lines (*CDH23*, *Myo7a*, *VLGR1*) have previously been linked to Usher syndrome, the leading cause of deaf-blindness in humans (*7*, *8*).

Slide 13: Usher syndrome

Usher syndrome has been classified into subtypes according to the severity and age of onset of symptoms. Usher syndrome type 1 (USH1) is the most severe form of the disease and is characterized by deafness from birth and progressive retinitis pigmentosa. Loss-of-function mutations in genes encoding the actin-binding molecular motor Myo7a, the adaptor proteins harmonin and SANS (scaffolding protein containing ankyrin repeats and a sterile alpha motif domain), or the transmembrane receptors cadherin 23 (CDH23) and protocadherin 15 (PCDH15) have been linked to USH1. Interestingly, partial loss-of-function mutations in some of these genes lead to nonsyndromic forms of deafness (DFNB, deafness autosomal recessive) and age-related hearing loss (ARHL). We have been particularly interested in CDH23 and PCDH15, which belong to the cadherin superfamily, the founding members of which mediate adhesive interactions between cells.

Slide 14: Cadherins and deafness

Whereas classical cadherins such as N- and E-cadherin contain five extracellular cadherin (EC) domains, CDH23 and PCDH15 contain 27 and 11 EC domains, respectively. We reasoned that transmembrane receptors with adhesive properties are good candidates to form some of the linkages that connect the stereocilia of a hair cell to one other, including the tip links, shown on the right in an electron micrograph of a bullfrog saccular hair cell.

Slide 15: High-resolution tip link structure

We therefore asked whether CDH23 and

PCDH15 might be tip-link components. High-resolution ultrastructural studies have shown that tip links consist of two strands that form a helical filament approximately 150 to 200 nm in length (*9*). The axial periodicity of tip links is 20 to 25 nm, with each strand being composed of globular structures that are each about 4.5 nm in diameter (*10*). The tip-link helix separates into at least two strands at both the upper and lower points of insertion into the stereocilia membranes.

Slide 16: Cadherin adhesion complexes

Crystallographic studies of classical cadherins reveal that the structure of the extracellular cadherin domain shows features resembling tip links. Like cadherins, tip links also connect two opposing membranes in a calcium-dependent manner. Some of the most revealing crystal structures are those obtained for C-cadherin, which mediates calcium-dependent homophilic adhesion between cells. The EC1 domains of C-cadherin mediate adhesive trans-interactions: A tryptophan side chain in the EC1 domain of one cadherin fits into a cavity of the EC1 domain of the opposing cadherin (*11*). These 10 EC domains of the C-cadherin adhesion complex span approximately 40 nm, and the structure has a slight bend. The much larger extracellular domains of CDH23 and PCDH15 might therefore form a helical structure that spans the 150- to 200-nm length of the tip link. Previous work has shown that CDH23 and PCDH15 are localized at the tip-link area of the immature hair cell bundle (*12*, *13*). However, it had been difficult to consistently detect these proteins in mature hair cells.

Slide 17: CDH23 distribution in adult rodent hair cells

To clarify the localization of CDH23 in mature hair cells, we generated several antibodies that recognize various epitopes in the extracellular domain and used them to reveal CDH23 localization in adult rodent cochlear hair cells. Hair bundles were labeled with rhodamine phalloidin (red) and immunostained with antibodies that recognize the CDH23 extracellular domain (green). CDH23 was confined to a region below the tips of stereocilia, consistent with localization at the upper insertion point of tip links (*14*). This experiment and others (*14*) suggest that CDH23 forms the upper part of the tip link.

Slide 18: PCDH15 distribution in adult rodent hair cells

In contrast, an antibody that recognizes the EC1 domain of PCDH15 stained the very tips of stereocilia, shown here in vestibular hair cells. The localization of PCDH15 suggested to us that it might be localized at the lower end of the tip links.

Slide 19: Tip-link model

The immunofluorescence localization studies are consistent with a model in which CDH23 forms the upper part of the tip link and interacts with PCDH15, which forms the lower part. To test this model, we used high-resolution immunogold localization studies. A total of three specific antibodies were used: one antibody that recognizes the EC1 domain of PCDH15, one that recognizes the linker region between the EC1 and EC2 domains of CDH23, and one specific for the linker region between the EC15 and EC16 domains of CDH23. If the EC domains of CDH23 and PCDH15 are similar in structure to the EC domains of classical cadherins, and if PCDH15 and CDH23 interact through their EC1 domains, then the distance between gold particles and the tips of stereocilia can be predicted as indicated in the figure.

Slide 20: Immunoelectron microscopy (immuno-EM) for CDH23 and PCDH15

Examples of immunogold localization using each of the antibodies are shown on the left. The distances between the gold particles and the stereocilia tips are consistent with our model of a tip link formed by CDH23 and PCDH15. The distribution of gold particles along the length of the tip link is shown on the right. The average distances from the lower tip-link insertion site to the immunogold-labeled domains [37 ± 17 nm for PCDH15-EC1 (n = 113); 52.5 ± 19 nm for CDH23-EC1/2 (n = 111); 138 ± 34 nm for CDH23-EC15/16 (n = 52)] closely agree with the predicted values and suggest that PCDH15 and CDH23 interact at their N termini to form tip links.

Slide 21: A novel tip-link model

These results suggest that a tip link is an adhesion complex formed by interactions between PCDH15 and CDH23. However, the immunogold localization studies do not yield information about the number of molecules required to form a tip link. Because tip links appear to be helical structures composed of two filaments, one hypothesis is that they might be formed by homodimers of CDH23 interacting in trans with PCDH15 homodimers. To test this model, we turned to a biochemical approach.

Slide 22: Purification of recombinant CDH23 and PCDH15

To determine whether CDH23 and PCDH15 molecules resemble tip links, we generated cDNAs encoding the extracellular domains of CDH23 and PCDH15 fused to His affinity tags and expressed each one separately in human embryonic kidney (HEK) 293 cells. The fusion proteins were purified with nickel–nitrilotriacetic acid (Ni-NTA) beads. Proteins were detected by Western blot analysis and silver staining.

Slide 23: Negative staining transmission electron microscopy (TEM)

Purified His-tagged CDH23 and PCDH15 extracellular domains were separately analyzed by negative staining TEM. In the presence of 1 mM Ca^{2+}, CDH23-His molecules formed homodimers with helical appearance. Frequently, the two strands of the dimers splayed at one end and formed a branched or looped structure. In the absence of Ca^{2+}, the CDH23 strands lost their filamentous shape, suggesting Ca^{2+}-dependant rigidification of the CDH23 extracellular domain. The PCDH15 extracellular domain also formed intertwined homodimers.

Slide 24: Parallel or antiparallel homodimers?

To define the orientation of the extracellular domains of CDH23 and PCDH15 in homodimers, we used Ni-NTA beads coupled to nanogold particles to label the base of the extracellular domain. Thus, parallel homodimers would be labeled at only one end, whereas antiparallel homodimers would be labeled at both ends. The beads bound only to the end of CDH23 homodimers that formed the branched or looped structures. Similar results were obtained for PCDH15-His homodimers, where gold beads attached to only one end of the homodimer. These findings suggest that the extracellular domains of CDH23 and PCDH15 form parallel homodimers. Furthermore, the measured lengths of the extracellular domains are in accordance with the prediction based on the number of EC domains (CDH23: 122 nm predicted, 129.9 ± 4.5 nm measured; PCDH15: 49.5 nm predicted, 51.9 ± 2.7 nm measured).

Slide 25: Interactions between CDH23 and PCDH15

The localization of CDH23 and PCDH15 at opposite tip-link ends suggests that they interact to form the tip links. To determine whether the two molecules can interact with each other, we analyzed a mixture of CDH23-His and PCDH15-His by TEM. Most molecules appeared as cis-homodimers, but we consistently observed complexes with the proper dimensions for CDH23 homodimers interacting in trans with PCDH15 homodimers (dotted circles mark the contact point between homodimers). The length of this complex is about 180 nm, which agrees with the reported length of tip links (150 to 200 nm). One end of the complex was frequently branched, which we infer to be the C-terminal

end of CDH23 (arrows). These findings provide further evidence that CDH23 and PCDH15 interact at their N termini and are consistent with additional biochemical data not presented here (*14*).

Slide 26: Molecular composition of tip links

Collectively, our findings provide evidence that tip links are asymmetrical adhesion complexes consisting of CDH23 and PCDH15 interacting at their N termini and forming the upper and lower part of tip links, respectively. Because the cytoplasmic domains of PCDH15 and CDH23 differ, this asymmetrical composition of tip links could reflect a general asymmetrical organization of the mechanotransduction machinery of hair cells, where distinct proteins with different functions are recruited to the upper and lower end of tip links.

Slide 27: The tip-link densities

By TEM, one observes that the two ends of each tip link are anchored at the stereociliary membrane in proximity to electron-dense plaques—the lower tip-link density (LTLD) and the upper tip-link density (UTLD)—marked by arrows in the micrograph. Tip-link densities are appropriately localized to contain additional components of the mechanotransduction machinery, including the transduction channel and molecules that link the channel to the cytoskeleton. Few proteins have been localized to LTLDs, and we do not know the composition of the UTLDs. Candidate components of tip-link densities are thus proteins that interact with the cytoplasmic domains of CHD23 and PCDH15.

Slide 28: CDH23- and PCDH15-interacting proteins

The cytoplasmic domains of CDH23 and PCDH15 are unrelated to one another, which suggests that each of the cadherins recruits a unique set of effectors. However, each does contain a C-terminal PDZ-binding motif. Using yeast two-hybrid analysis and protein interaction studies, we and others identified the adaptor protein harmonin as a binding partner for CDH23, PCDH15, F-actin, and harmonin itself (*15*–*18*). Harmonin is composed of three PDZ domains, two coiled-coil (CC) domains, and a proline-serine-threonine–rich (PST) domain. High-affinity interaction between harmonin and cadherins occurs through harmonin's PDZ2 domain. The region containing the CC, PST, and PDZ3 domains interacts with F-actin. Both harmonin and PCDH15 also can bind to MYO7A, which in turn binds to F-actin (*19*).

Slide 29: Usher syndrome

Null mutations in *MYO7A* and *USH1C* (which encodes harmonin), like those in *CDH23* and *PCDH15*, lead to Usher syndrome. Null mutations in the orthologous mouse genes also cause deafness characterized by splaying of the stereociliary bundles, which suggests that the auditory phenotype in Usher syndrome patients is caused by defects in the development or maintenance of hair bundles. This interpretation is consistent with the distribution of the Usher proteins during hair cell development. Hypomorphic mutations in some of the Usher genes lead to less severe forms of hearing impairment (*20*). We therefore wondered whether defects at tip links could cause the less severe forms of the disease. One prediction of this hypothesis is that Usher proteins, such as harmonin, are localized at tip links.

Slide 30: Harmonin localization in hair cells

To define the expression pattern of harmonin in hair cells, we generated an antibody specific for the PDZ3 domain of harmonin. In developing hair cells, harmonin (shown in red) was confined to a region below the tips of stereocilia, similar to the distribution to CDH23 and the UTLD.

Slide 31: Harmonin localizes to the UTLD

To determine whether harmonin is a genuine component of the UTLD and present in mature hair cells, we analyzed its localization by immunogold labeling and TEM in outer hair cells of postnatal day 35 (P35) animals. We quantified the distribution of gold particles in 16 stereocilia pairs where the plane of sectioning revealed tip-link densities. Most of the gold particles (69%) were localized at the UTLDs. The other particles were distributed over the much larger remaining surface area of the stereocilia. Few gold particles were detected at LTLDs.

Slide 32: Harmonin and tip links

We conclude that harmonin is present in functional, mature hair cells, where it is concentrated at UTLDs. This localization is reminiscent of the asymmetric distribution of CDH23, which is located at the upper end of tip links.

Slide 33: CDH23 and harmonin in hair cells

We wondered whether harmonin might connect CDH23 to the F-actin cytoskeleton. We therefore analyzed mice with mutations in harmonin that disrupt its interactions with either CDH23 or F-actin. We found that a mutation in PDZ2 that disrupts interactions between CDH23 and harmonin prevents proper harmonin localization in hair cells and disrupts hair bundle morphogenesis (*20*). Next, we analyzed deaf circler (*dfcr*) mice (*21*), which carry an in-frame deletion in *harmonin* that removes the exons encoding the CC and PST domains. This mutation is predicted to disrupt interactions between harmonin and F-actin.

Slide 34: Localization of harmonin in dfcr *mice*

Hair bundle morphology was unaffected in *dfcr* mice during the first few months after birth, but ABR recordings demonstrated that homozygous *dfcr* mice were deaf by the age of 4 weeks, with an auditory threshold of >90 dB. Moreover, harmonin immunoreactivity in cochlear hair cells of homozygous *dfcr* mice shifted to the very tips of stereocilia. These results suggest that the CC and PST domains of harmonin are required for its localization in hair bundles and for normal hearing function.

Slide 35: UTLDs in dfcr *mice*

Because the CC and PST domains of harmonin are not essential for hair bundle development, we asked whether they might function at UTLDs. Using TEM, we examined UTLDs in homozygous *dfcr* mice at P10, when UTLDs can first be easily detected in wild-type animals, and at P18 and P70, when UTLDs have assumed their characteristic cup-shaped appearance (black arrowheads). At all stages analyzed, we never detected electron-dense structures resembling the UTLDs in *dfcr* mutants, even though LTLDs were present. We conclude that harmonin is a component of the UTLDs, and that its CC and PST domains are required for the formation or stability of UTLDs. In our TEM experiments, we also detected tip links (red arrowhead) in *dfcr* mutants, indicating that harmonin is not required for establishing or maintaining these structures. The presence of tip links was further confirmed by scanning electron microscopy (SEM), as shown in the pictures on the right where white arrowheads mark tip links.

Slide 36: Mechanotransduction currents in dfcr *mice*

Because hair bundle morphology was not noticeably affected in *dfcr* mice, hair cells from these mutants were ideally suited to determine the effect of *harmonin* mutations on the mechanotransduction properties of cochlear hair cells. We measured transducer currents in P7–P8 cochlear hair cell explants using whole-cell recordings from cells stimulated with a stiff glass probe (diagram on left). Representative current traces from single hair cells are shown at the top right, with an analysis of the results from several recordings (n = 10 for controls, n = 16 for mutants) shown below. Mechanotransduction currents of similar magnitude could be evoked in hair cells from

control (*dfcr* heterozygous) and *dfcr* homozygous mice. However, when the open probability of the transduction channel (P_o) was plotted as a function of the magnitude of the hair displacement, it was apparent that the resulting curve derived from the mutants was significantly broadened and shifted to the right relative to the control. These findings indicate that the gating properties of mechanotransduction channels are altered in *dfcr* mice, where the sensitivity of the hair bundle to displacement is reduced.

Slide 37: Adaptation in hair cells from dfcr *mice*

Following activation, transducer currents adapt in a biphasic response, where fast adaptation in cochlear hair cells occurs within less than 1 ms, and slow adaptation in several milliseconds. Fast adaptation is thought to be caused by binding of Ca^{2+} to the channel pore itself or to a site near the pore, whereas slow adaptation is thought to depend on the motor protein MYO1C. MYO1C is thought to control the position of the transduction complex along the length of the stereocilium. As a result of adaptation, the P_o of a channel is reset toward its resting value, which is near the point of optimal sensitivity along the displacement-P_o curve.

We reasoned that the rightward shift and change in shape of the displacement-P_o curve in *dfcr* mice might result from altered adaptive properties. We therefore determined the effects of the mutation on resting P_o and adaptation. The resting P_o of *dfcr* mutants was 1.3% ± 0.3%, significantly lower than that of control animals (3.4% ± 0.6%,). We also plotted the time constants for adaptation against P_o and observed that the rates of both fast and slow adaptation were significantly reduced in *dfcr* mice relative to controls. We conclude that harmonin is critical for normal adaptation by hair cells.

Slide 38: The tip-link complex

Our studies provide evidence that CDH23 and PCDH15 form tip links in hair cells and that harmonin is localized to the upper ends of tip links, where it is required for normal gating of transduction channels by mechanical force. Because MYO1C and harmonin are both concentrated at the upper ends of tip links, it is possible that harmonin plays a role in controlling resting tension and adaptation by regulating the activity of the MYO1C motor complex and its coupling to the cytoskeleton. In *dfcr* mice, defects in the function of the MYO1C motor complex therefore affect channel gating and adaptation. Our findings, as well as the recent studies by Ricci and colleagues (*3*), also reinforce the concept that the mechanotransduction machinery of hair cells is structurally and functionally asymmetric.

Slide 39: Mechanotransduction machineries

Coming back to the general blueprint of mechanotransduction machineries, it is interesting to note that completely different components have been identified as required in *C. elegans* touch-sensitive neurons and mammalian hair cells, which suggests that at least two mechanotransduction machineries arose during evolution.

Slide 40: Acknowledgments

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